Construction of T-vectors, a rapid and general system for direct cloning of unmodified PCR products

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Submitted December 19, 1990

Although the polymerase chain reaction (1, 2) (PCR) can be used to produce a large amount of a specific DNA from a complex source, cloning the PCR products has not proven to be straightforward. Restriction endonuclease sites are often incorporated into the oligonucleotide primers used for amplification, so that cleavage of the product will create sticky ends that can theoretically be ligated to an equivalently cut vector (3). Unfortunately, many restriction endonucleases fail to cleave when their recognition sequences are located within a few base pairs of the end of a DNA fragment (4, 5). Ligation-independent PCR cloning schemes (6, 7, 8) involve the addition of at least 12 bases to the 5′ end of the primer, which can increase the cost of synthesis substantially when done routinely. Attempts to clone PCR products as blunt-ended fragments have been very inefficient, due to the template-independent terminal transferase activity of Taq polymerase, which results in the addition of a single nucleotide at the 3′ end of the fragment (9, 10). This nucleotide is almost exclusively an adenosine, due to the strong preference of the polymerase for dATP (9). Thus, cloning the products as blunt-ended fragments requires enzymatic processing to remove of the 3′ overhang using an enzyme with 3′ to 5′ exonuclease activity (11).

This template-independent activity of Taq polymerase can be exploited to create a cloning scheme which has the efficiency of sticky end cloning, but requires no additional enzymatic modification of the PCR product. Bluescript (Stratagene, La Jolla, CA) plasmid is digested with EcoRV, and incubated with Taq polymerase (1 unit/ μg plasmid/20 μl volume) using standard buffer conditions (50 mM KCl, 10mM Tris pH 8.3, 1.5 mM $MgCl_2$, and 200 μg/ml BSA) in the presence of 2 mM dTTP for 2 hours at 70°C. The absence of any other nucleotides in the reaction results in the addition of a single thymidine at the 3′ end of each fragment. After phenol extraction and precipitation, the T-vector is ready for cloning. PCR products are gel purified to avoid cloning spurious bands, and ligated to the vector at 14°C. The vector and PCR products have complementary single base 3′ overhangs. Vector self-ligation events are prohibited by the 3′ thymidine overhang, and concatamerization of the insert is prohibited by the unphosphorylated 5′ end, contributed by the oligonucleotide primer, as well as the 3′ adenosine overhang added by Taq polymerase during the PCR reaction. The use of the Bluescript plasmid allows the blue versus white color selection for insert containing clones, since a small percentage of vector molecules escape thymidine addition. Sequencing of the insert containing clones reveals the insertion of a single T:A base pair between the EcoRV site and the PCR primer sequence. This procedure is at last one hundred fold more efficient that attempting to clone the PCR products into a blunt-ended cut vector.

The 3′ overhangs of PCR products have been exploited in a commercially prepared cloning system (TA Cloning kit of Invitrogen, San Diego, CA). However, the simple procedure outlined here can be readily adapted to any plasmid or viral DNA based vector with a unique blunt-ended restriction endonuclease site. The procedure is very general so that a single batch of prepared vector can be used for cloning any PCR product without having to redesign oligonucleotide primers. Nine of nine different PCR products were cloned into a plasmid T-vector on the first attempt. Additionally, it will find more general use in cloning schemes where two DNA fragments with incompatible sites need to be joined together. Fragments can be made blunt-ended by filling in using Klenow (5′ overhang) or trimmed with T4 DNA polymerase (3′ overhang). One fragment can subsequently be treated with Taq polymerase in the presence of dATP, the other in the presence of dTTP, creating complementary one base pair sticky ends for more efficient ligation.

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